L-02-0017
Study 1

Acute Dermal Toxicity Study in Rats (970593); March 24, 1998

REDACTED AS TO TRADE NAMES

LABORATORIES & CLINICS GROUP
BIOSCIENCE

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ACUTE DERMAL TOXICITY STUDY IN RATS

RBM EXP. No. 970593

EEC Guidelines (B.3)
OECD Guidelines (402)

Issued on March 24, 1998

SPONSOR

AUSIMONT
Viale S. Pietro, 50/A
20021 BOLLATE (Milano)
Italy

PERFORMING LABORATORY

Istituto di Ricerche Biomediche
"Antoine Marxer" RBM S.p.A.
Via Ribes, 1
10010 - COLLERETTO GIACOSA (Torino)
Italy

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RBM Exp. No. 970593

TITLE OF THE STUDY

"Acute dermal toxicity study in rats treated with the test article [redacted]"

PURPOSE OF THE STUDY

The purpose of the study was to evaluate the acute dermal toxicity of the test article [redacted].

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This report consists of 48 pages.

Ivrea, March 24, 1998

Dr. Ping Yu
RBM Study Director

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RBM Exp. No. 970593

FOREWORD

On behalf of **AUSIMONT - Viale S. Pietro, 50/A, 20021 BOLLATE Milano - Italy** - Istituto di Ricerche Biomediche "Antoine Marxer" RBM S.p.A., authorized by the Italian Health Authorities (1-2) to conduct safety studies, has performed an acute toxicity study by dermal route in Sprague Dawley Crl: CD(SD) BR rat (RBM-Experiment No. 970593), with the test article:

[redacted]

A sample of the substance used, along with pertinent documentation, is held in sufficient quantity in the RBM archives and is at the disposal of the Ministero della Sanità.

The undersigned declare that the experiment was conducted using the same batch of substance as that of the sample held on file.

For verification by the Ministero della Sanità, the undersigned moreover guarantee the identification and classification of all those materials, documents and recordings used in conducting the experiment, held on file for a period of at least 10 years from the date of this report. Following this time, they will be placed at the disposal of the Sponsor.

Dr. Roberto Maraschin

Scientific Director Recognized by the Italian Health Authorities as Responsible for General Toxicology Experimentation

Dr. Angelo Conz

General Manager of the Istituto di Ricerche Biomediche "Antoine Marxer" , RBM S.p.A.

Ivrea, March 24, 1998

(1): **Pharmaceuticals:**
Authorization dated March 12, 1976 in accordance with "Circolare 73", May 16, 1974

(2): **Chemicals:**
Authorization in accordance with DPR 927/81 (D.M. dated January 7, 1988 published in G.U. No. 12, dated January 16, 1988).

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RBM Exp. No. 970593

QUALITY ASSURANCE STATEMENT

RBM Experiment number: 970593

Study title:

"Acute dermal toxicity study in rats treated with the test article [redacted]"

Studies of the type described in this report are conducted in a manner which involves frequent repetition of identical or similar procedures.

In compliance with the Principles of Good Laboratory Practice, at the time of this study, procedure-based inspections were made by the Q.A.U. of critical phases and procedures relevant to this type of study. For the inspection of any given procedure, studies were selected at random. All such inspections were reported promptly to the study director and to facility management.

Dates of inspection/audit	Dates of report to Study Director and Management
January 13, 1998 March 20 - 23, 1998	January 13, 1998 March 23, 1998

This report has been audited by the Q.A.U. and was found to be an accurate description of such methods and procedures as were used during the conduct of the study and an accurate reflection of the raw data.

Date of final report audit: March 23, 1998

Date : March 23, 1998

Enrico Invernizzi
Head of Quality Assurance Unit

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RBM MANAGEMENT DECLARATION OF GLP COMPLIANCE

Study No. 970593 entitled :

"Acute dermal toxicity study in rats treated with the test article [redacted]"

was performed in compliance with the OECD-GLP in the testing of chemicals, [C(81) 30 (final)], regulations also enforced by the Italian Health Authority [D.M. dated June 26, 1986 as published in G.U. No. 198, dated August 27, 1986 and D.L. January 27, 1992, No. 120 as published in G.U. (Supplement) No. 40, February 18, 1992].

Dr. Ping Yu
RBM Study Director

Dr. Angelo Conz
General Manager of the Istituto di Ricerche Biomediche "Antoine Marxer", RBM S.p.A.

Ivrea, March 27, 1998

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SCIENTISTS INVOLVED IN THE STUDY

STUDY No. 970593

"Acute dermal toxicity study in rats treated with the test article [redacted]"

RBM Study Director	Dr. Ping Yu
Scientific Director Toxicology	Dr. Roberto Maraschin
Head of General Toxicology I Unit	Dr. Germano Oberto

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MATERIALS AND METHODS

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EXPERIMENTAL DESIGN

RBM Experiment No.:	970593
Test article:	[redacted]
Administration route:	epidermal
Exposure period:	about 24 hours
Duration of treatment period:	single administration
Duration of post-treatment observation period:	14 days after the 24-hour exposure period

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.3) and with Organization for Economic Cooperation and Development Guidelines (section 4, subpart 402, Paris 1981 and subsequent revisions).

TEST SYSTEM

Species, strain and substrain:	Sprague Dawley Crl: CD (SD) BR rat
Justification for selection of the test system :	the Sprague Dawley rat was chosen as rodent species since it is an appropriate experimental model widely accepted by Health Authorities, with documented susceptibility to a wide range of toxic substances
Dosages administered	2000 mg/kg in 5 males and 5 females 1000, 500 and 200 mg/kg in 5 males/dose

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Body weight (at randomization):	Males: 230 - 304 g Females: 221 - 261 g
Age (at randomization):	no more than three months
Supplier:	Charles River Italia S.p.A. Via Indipendenza, 11 22050 CALCO (Lecco) Shipping slips No.s 8504 (December 12, 1997), 8353 (December 5, 1997), 597 (January 23,1998) and 793 (January 30,1998)
Acclimatation:	more than 5 days before the start of the test. Animals were observed daily to ascertain their fitness for the study.
Housing:	5 animals/sex/cage in air-conditioned room. - Temperature: 22°C ± 2 - Relative humidity: 55% ± 10 - Air changes: about 20 / hour filtered on HEPA 99.97% - Light: 12 hour cycle (7 a.m. - 7 p.m.) - Cage size: grill cages 40.5x38.5x18h cm with stainless steel feeder. The waste that dropped through the grill bottom onto removable paper was periodically disposed of.
Animal identification:	by appropriately coloring different areas of the limbs. Cage card gave experiment number, dosage group, sex and date of administration.
Diet:	GLP 4RF21 top certificate pelleted diet produced by Charles River Italia's feed licencee Mucedola S.r.l., Settimo Milanese. The declare contents, on the label, on dry matter basis (moisture 12%), were:

crude protein	18.50%
crude fat	3.00%
crude fiber	6.00%
crude ash	7.00%

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The diet was supplemented by the Producer with vitamins and trace elements. The Producer supplies a certificate of analysis for nutrients and contaminants, the levels of which are within the limits proposed by EPA-TSCA (44FR:44053-44093, July 26, 1979).
RBM has the animal feed re-analyzed at least twice a year for bacterial contamination.
The diet was available "ad libitum" to the animals.

Water: from the municipal water main system.
Water is filtered and distributed "ad libitum" to the animals by an automatic valve system.
Periodically drinking water is analyzed for microbial count, heavy metals, other contaminants (e.g. solvents, pesticides) and other chemical and physicals characteristics. The accepted limits of quality of the drinking water were those defined in EEC directive 80/778

Contaminants that might interfere with the objectives of the study were not expected to be present in diet or drinking water.

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TEST ARTICLE IDENTIFICATION, CHARACTERIZATION AND FORMULATE

The test article was supplied by the Sponsor as follows:

Identification:	[redacted]
Batch:	19387/20
Characteristics:	white solid
Purity:	>99%
Manufacturing date:	December, 1997
Expiry date:	December, 2000
Storage conditions:	at room temperature

TEST DESCRIPTION

Administration route: epidermal

Reason for selection of administration route: possible accidental exposure in humans

Experimental design:

Dose mg/kg		Treatment date	Final killing
2000	males:	January 15, 1998	found dead
2000	females*:	January 23, 1998	found dead
1000	males:	February 6, 1998	February 28, 1998
500	males	February 27,1998	March 14, 1998
200	males	February 27, 1998	March 14, 1998

* 5 females were treated at the dose of 2000 mg/kg since there were no clinical signs observed in the males given the same dose during the first days of treatment.

Preparation of animals skin: approximately 24 hours before the test, fur was clipped from the dorsal and ventral area of the trunk of the test animals. Care was taken to avoid abrading the skin which could alter its permeability.
An area of about 6x5 cm of the body dorsal surface was cleared for the application of the test article.
This area corresponded to about 10% of the total body surface.

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Administration of the test article:	the test article was applied uniformly onto a porous gauze which was moistened with 0.9% NaCl. The treated area was covered with the porous gauze dressing fixed to the skin with hypoallergenic non-irritating tape. The test site was further covered in a suitable manner in order to ensure that the animals could not ingest the test substance. At the end of the exposure period the residual test article was wiped off with water.
Observation period:	14 days (for the 500 and 200 mg/kg groups) or 22 days (for the 1000 mg/kg group) after the 24-hour exposure period. All animals of the 2000 mg/kg group died within 15 days of dosing.
Observation of clinical signs and mortality:	at 30 minutes, 2, 4 and 6 hours on the first day after the administration (day 1) and then twice a day up to termination of the observation period.
Body weight:	twice pre-trial (at randomization and on day 1 just before administration) and on days 8, 15 and/or 22. Volume of administration was based on day 1 body weight.
Gross pathology:	on animals which died before the end of the study and on animals (fasted overnight) killed by excision of the femoral arteries, after i.p. overdosage anesthesia with 5% sodium pentobarbital, at the end of the observation period
Histology:	Histologic examination was not performed.
LD_{50} and its statistical limits:	LD_{50} was calculated by the method of the Probit (Bliss - Finney) - A.P. Rosiello et al., J. Tox. and Env. Health, 3: 797-809, 1977.

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RECORD FILING

The protocol, a reserve sample of the test article used, the raw data bound in a register numbered 970593/1, the final report and all other documents pertinent to the conduct of this study, including records and reports of maintenance, cleaning, calibration and inspection of equipment, analysis of diet and water are filed at RBM premises for ten years from the issue date of this report and then sent to the Sponsor.

PROCEDURAL DETAILS

The study was conducted in accordance with the procedures described in the RBM Standard Operating Procedures (SOP's) collection.

Protection of animals used in the experiment is in accordance with Directive 86/609/EEC, enforced by the Italian D. L. No. 116 of January 27, 1992.

Physical facilities and equipment for accomodation and care of animals are in accordance with the provisions of EEC Council Directive 86/609.

The Institute is fully authorized by Competent Veterinary Health Authorities.

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RESULTS

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CLINICAL OBSERVATIONS

MORTALITY *(TABLE 1)*

The deaths which occurred in the various dose groups are shown below:

Dose (mg/kg)	200	500	1000	2000
Treated animals	5 M	5M	5M	5M + 5F
Mortality	0	2M	4M	5M+5F
Total (%)	0%	40%	80%	100%

The deaths occurred within 18 days of treatment, with the first case observed on 7 days after dosing in one male of the 2000 mg/kg group.

The LD_{50} was calculated to be 600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.

CLINICAL SIGNS *(TABLE 2 AND APPENDIX 1)*

Hypoactivity, piloerection, hunched posture, skin and mucosae pallor and hypothermia were observed in animals of the higher dose groups (500 - 2000 mg/kg), starting on days 6-7 after dosing at 2000 mg/kg and on days 8-15 after dosing at the lower doses. Some animals of the highest dose group (2000 mg/kg) also showed sedation and perineum stained with urine.

In addition, changes at the treatment site including skin edema and erythema were found in animals of the 2000 mg/kg group.

Recovery of the clinical changes in the surviving animals was achieved by day 13 (500 mg/kg group) or by day 21 (1000 mg/kg group) of the observation period.

No changes of note were seen in animals given the test article at the lowest dose (200 mg/kg).

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BODY WEIGHT *(APPENDIX 2)*

Decrease in body weight was found in animals of the higher dose groups (2000 and 1000 mg/kg) during the study period. Body weights of animals in the lower dose groups were found to be unaffected by the test article administration.

POST-MORTEM EXAMINATION

GROSS PATHOLOGY *(TABLE 3 AND APPENDIX 3)*

At the autopsy of animals which died before the end of the observation period the macroscopic findings were liver paleness (2000 mg/kg group) or liver increased size (1000 and 500 mg/kg groups), congestion of stomach, decreased size and/or paleness of spleen and kidney medulla congestion. Moreover, skin edema (treatment site) was found in animals of the 2000 mg/kg group.

At the final killing increased size of liver was seen in animals of the 500 mg/kg group. No appreciable modifications were found in animals of the 200 mg/kg group.

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SUMMARY AND CONCLUSIONS

Experimental data from an acute toxicity study in which Sprague Dawley Crl:CD(SD) BR rats were treated by dermal route with the test article [redacted] are given in this report.

The test method was in accordance with European Economic Community Guidelines - Annex to Commission Directive 92/69/EEC of July 31, 1992 adapting to technical progress for the seventeenth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (B.3) and with Organization for Economic Cooperation and Development Guideline (section 4, subpart 402, Paris 1981 and subsequent revisions).

The test article was applied uniformly onto a porous gauze which was moistened with 0.9% NaCl and then, this porous gauze was fixed to the dorsal and ventral area of trunk of the rats (fur was clipped 24 hours previously). The individual dosages were based on body weight taken just before treatment.

The day of treatment was considered day 1 of the study. The animals were weighed twice before treatment (at randomization and on day 1 just before treatment) and on days 8, 15 and/or 22. They were clinically observed for 14 days (for the 200 and 500 mg/kg groups) or 22 days (for the 1000 mg/kg group; all 2000 mg/kg rats died within 15 days) after the 24-hour exposure period. Necropsy examination was performed on all animals which died before the end of the study. On day 16 or day 23 the surviving rats were killed (fasted overnight) by excision of the femoral arteries after i.p. overdosage anesthesia with 5% sodium pentobarbital and were submitted to a thorough autopsy.

The deaths which occurred in the various dose groups are showen below:

Dose (mg/kg)	200	500	1000	2000
Treated animals	5 M	5M	5M	5M + 5F
Mortality	0	2M	4M	5M+5F
Total (%)	0%	40%	80%	100%

The deaths occurred within 18 days of treatment, with the first case observed on 7 days after dosing in one male of the 2000 mg/kg group.

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The LD_{50} was calculated to be 600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.

Hypoactivity, piloerection, hunched posture, skin and mucosae pallor and hypothermia were observed in animals of the higher dose groups (500 - 2000 mg/kg), starting on days 6-7 after dosing at 2000 mg/kg and on days 8-15 after dosing at the lower doses. Some animals of the highest dose group (2000mg/kg) also showed sedation and perineum stained with urine. In addition, local changes including skin edema and erythema (treatment site) were found in animals of the 2000 mg/kg group.

Recovery of the clinical changes in the surviving animals was achieved by day 13 (500 mg/kg group) or by day 21 (1000 mg/kg group).

No changes of note were seen in animals given the test article at the lowest dose (200 mg/kg).

Decrease in body weight was found in animals of the higher dose groups (2000 and 1000 mg/kg) during the study period. Body weights of animals in the lower dose groups were found to be unaffected by the test article administration.

At the necropsy of animals which died before the end of the observation period, the main macroscopic findings were liver paleness (2000 mg/kg group) or liver increased size (1000 and 500 mg/kg groups). Moreover, skin edema (treatment site) was found in animals of the 2000 mg/kg group.

At the final killing, increased size of liver was seen in animals of the 500 mg/kg group. No appreciable modifications were found in animals of the 200 mg/kg group.

In conclusion, the **LD_{50}** of the test article [redacted], when administered by dermal route to the rats, was **600 mg/kg with 95% confidence limits of 414 - 871 mg/kg.**

The compound induced delayed toxicity (liver was mainly involved) and local changes (treatment site) which were confined to the animals treated at the higher doses.

Dr. Ping Yu

RBM Study Director

March 24, 1998

Dr. Roberto Maraschin

Scientific Director Recognized by the Italian Health Authorities as Responsible for General Toxicology Experimentation

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GROUP DATA

[redacted] 160

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 1. - Mortality and LD50 calculation (p. 1)

Males - Females

Dose (mg/kg)	200	500	1000	2000
Treated animals	5	5	5	10
Day 7	0	0	0	1
8	0	0	0	1
9	0	0	0	1
10	0	0	0	1
12	0	0	0	1
13	0	2	0	1
14	0	0	0	2
15	0	0	1	2
18	0	0	3	0
Total no. (day 22)	0	2	4	10
Total (%)	.0%	40.0%	80.0%	100.0%

Median lethal dose (LD50) = 600.30

95% confidence limits = 413.92 - 870.61

Slope (SE) = 1.91 .51

Heterogeneity P = .959 NS

Linear regression y =-7.1954+1.9063x

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 1)
(no. of animals affected, from-to)

Males

Dose (mg/kg)	200	500	1000	2000
no. of treated animals	5	5	5	5
Death	-	2 13d	4 15d-18d	5 7d-14d
Sedation	-	-	-	2 7d- 8d
Hypoactivity	-	2 11d-12d	5 8d-17d	3 6d-13d
Piloerection	-	2 11d-12d	5 8d-20d	3 6d-13d
Hunched posture	-	2 11d-12d	5 8d-20d	3 6d-13d
Skin and app. mucosae, pallor	-	2 12d-12d	4 15d-18d	2 6d-13d
Hypothermia	-	2 12d-12d	4 15d-17d	2 6d-13d
Skin treatment site: edema	-	-	-	5 2d-13d

- (not observed) from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 2)
(no. of animals affected, from-to)

Males

Dose (mg/kg)	200	500	1000	2000
no. of treated animals	5	5	5	5
Skin treatment site: erythema	-	-	-	3 6d- 9d
Perineum stained with urine	-	-	-	1 6d- 6d
Recovery	-	3 13d	1 21d	-

[redacted]

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- (not observed) from-to (first-last observation in one or more animals)
Time : d (days)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 3)
(no. of animals affected, from-to)

Females

Dose (mg/kg)	2000
no. of treated animals	5
Death	5 8d-15d
Sedation	1 12d-12d
Hypoactivity	5 6d-14d
Piloerection	5 6d-14d
Hunched posture	5 6d-14d
Skin and app. mucosae, pallor	1 7d- 9d
Hypothermia	1 7d- 9d
Skin treatment site: edema	5 2d-13d

from-to (first-last observation in one or more animals)
Time : d (days)

[redacted]

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 2. - Clinical signs (maximum daily frequency) (p. 4)
(no. of animals affected, from-to)

Females

Dose (mg/kg)	2000
no. of treated animals	5
Skin treatment site: erythema	1 7d-10d
Perineum stained with urine	2 6d-14d

[redacted]

from-to (first-last observation in one or more animals)
Time : d (days)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 3. - Gross pathology examination (p. 1)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	200	500	1000	2000
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	0	0	0
General observation				
cannibalized	-	0	1 25.00%	0
Kidneys				
medulla, congestion	-	0	3(2.3) 75.00%	3(2.0) 60.00%
Liver				
increased size	-	2(2.5) 100.00%	3(2.3) 75.00%	0
pale	-	0	0	5(2.8) 100.00%
Skin treatment area				
edema	-	0	0	4(2.0) 80.00%

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 3. - Gross pathology examination (p. 2)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an. Males

Dose (mg/kg)	200	500	1000	2000
no. of animals	0	2	4	5
no. of animals without appreciable lesions	0	0	0	0
Spleen				
decreased size	-	0	3(2.0) 75.00%	5(2.0) 100.00%
Stomach				
congestion	-	0	2(2.0) 50.00%	0

[redacted]

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 3. - Gross pathology examination (p. 3)
(no. of cases, mean severity, %)

Final killing	Males			
Dose (mg/kg)	200	500	1000	2000
no. of animals	5	3	1	0
no. of animals without appreciable lesions	5	0	0	0
Liver				
increased size	0	3(2.0) 100.00%	1(2.0) 100.00%	-

- (not examined)
Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

TABLE 3. - Gross pathology examination (p. 4)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an.	Females
Dose (mg/kg)	2000
no. of animals	5
no. of animals without appreciable lesions	0
Kidneys	
medulla, congestion	3(2.0) 60.00%
Liver	
pale	5(2.6) 100.00%
Skin treatment area	
edema	2(2.0) 40.00%
Spleen	
decreased size	3(2.7) 60.00%

[redacted]

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Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
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TABLE 3. - Gross pathology examination (p. 5)
(no. of cases, mean severity, %)

Dead or agonal sacrificed an.	Females
Dose (mg/kg)	2000
no. of animals	5
no. of animals without appreciable lesions	0
Stomach	
congestion	1(2.0) 20.00%

[redacted]

Severity : 0(very slight) 1(slight) 2(moderate) 3(severe)

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APPENDICES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 1)
(no. of animals affected)

Dose (mg/kg) 200

Cage # 7M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Time: m (minutes) h (hours) M (morning) A (afternoon)

[redacted]

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 2)
(no. of animals affected)

Dose (mg/kg) 500

Cage # 5M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																												2					
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	3	3	3	3	3	3	3	3	3
Hypoactivity																								2	2	2	2						
Piloerection																								2	2	2	2						
Hunched posture																								2	2	2	2						
Skin and app. mucosae, pallor																										2	2						
Hypothermia																										2	2						

[redacted]

Time: m (minutes) h (hours) M (morning) A (afternoon)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 3)
(no. of animals affected)

Dose (mg/kg) 1000

Cage # 3M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17	
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Death																														1					
No clinical signs		5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	1	1																
Hypoactivity																		1	1	1	1	3	3	5	5	5	5	5	5	5	5	4	4	4	4	4	4
Piloerection																		4	4	4	4	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4
Hunched posture																		4	4	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4
Skin and app. mucosae, pallor																																4	4	4	4	4	4
Hypothermia																																4	4	4	4	3	3

Cage # 3M (follows)	Day	18		19		20		21		22	
	Time	M	A	M	A	M	A	M	A	M	A
Death		3									
No clinical signs								1	1	1	1
Piloerection		1	1	1	1	1	1				
Hunched posture		1	1	1	1	1	1				
Skin and app. mucosae, pallor		1	1								

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 4)
(no. of animals affected)

Dose (mg/kg) 2000

Cage # 1M	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Death																1				1						1				2
No clinical signs		5	5	5	5																									
Sedation																2	2	1	1											
Hypoactivity														1	1			1	1	1	1	3	3	3	3	2	2	2	2	
Piloerection														1	1	2	2	2	2	2	2	3	3	3	3	2	2	2	2	
Hunched posture														2	2	2	2	2	2	2	2	3	3	3	3	2	2	2	2	
Skin and app. mucosae, pallor														1	1									1	1			2	2	
Hypothermia														1	1									1	1			2	2	
Skin treatment site: edema						5	5	5	5	5	5	5	5	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Skin treatment site: erythema														3	3	2	2	2	2	1	1									
Perineum stained with urine														1	1															

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 1. - Clinical signs incidence (p. 5)
(no. of animals affected)

Dose (mg/kg) 2000

Cage # 2F	Day	1				2		3		4		5		6		7		8		9		10		11		12		13		14		15
	Time	30m	2h	4h	6h	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M
Death																		1				1						1				2
No clinical signs		5	5	5	5																											
Sedation																										1	1					
Hypoactivity														1	1	5	5	4	4	4	4	3	3	3	3	2	2	2	2	2	2	
Piloerection														2	2	5	5	4	4	4	4	3	3	3	3	3	3	2	2	2	2	
Hunched posture														1	1	5	5	4	4	4	4	3	3	3	3	2	2	2	2	2	2	
Skin and app. mucosae, pallor																1	1			1	1											
Hypothermia																1	1			1	1											
Skin treatment site: edema						5	5	5	5	5	5	5	5	5	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Skin treatment site: erythema																1	1	1	1	1	1	1	1									
Perineum stained with urine														2	2	2	2	1	1	2	2	1	1	1	1	2	2	2	2	2	2	

Time: m (minutes) h (hours) M (morning) A (afternoon)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 2. - Body weight (g) (p. 1)
(individual)

Dose (mg/kg) 200

Week	day	Animal # 31M	32M	33M	34M	35M
	0	230	230	230	230	230
1	1	235	231	232	230	231
2	8	295	291	298	290	286
3	15	349	329	320	326	315

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 2. - Body weight (g) (p. 2)
(individual)

Dose (mg/kg) 500

Week	day	Animal # 21M	22M	23M	24M	25M
	0	236	232	230	232	234
1	1	241	236	234	237	238
2	8	278	253	269	255	254
3	15		278	294	269	

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 2. - Body weight (g) (p. 3)
(individual)

Dose (mg/kg) 1000

Animal #		11M	12M	13M	14M	15M
Week	day					
	0	295	296	294	272	250
1	1	300	308	310	280	286
2	8	228	258	227	215	263
3	15	167	177	167		188
4	22					261

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 2. - Body weight (g) (p. 4)
(individual)

Dose (mg/kg) 2000

Animal #		1M	2M	3M	4M	5M	6F	7F	8F	9F	10F
Week	day										
	0	296	289	270	286	304	237	261	221	222	222
1	1	302	294	279	285	310	249	258	225	216	229
2	8	233	235	191	239		185		155	201	170

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 1)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 500

An#	Death day/code#	TISSUE	Gross observations
21M	13 M2	Liver	increased size, diffuse, severe
25M	13 M2	Liver	increased size, diffuse, moderate

Death code : M2 (Natural death)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 2)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 1000

An#	Death day/code#	TISSUE	Gross observations
11M	18 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	increased size, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate
12M	18 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	increased size, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
13M	18 M2	General observation	cannibalized
14M	15 M2	Kidneys	medulla, congestion, diffuse, severe
		Liver	increased size, diffuse, severe
		Spleen	decreased size, diffuse, moderate
		Stomach	congestion, diffuse, moderate

Death code : M2(Natural death)

REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 3)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#	TISSUE	Gross observations
1M	14 M2	Liver	pale, diffuse, severe
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
2M	12 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, severe
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
3M	9 M2	Kidneys	medulla, congestion, diffuse, moderate
		Liver	pale, diffuse, moderate
		Skin treatment area	edema, diffuse, moderate
		Spleen	decreased size, diffuse, moderate
4M	14 M2	Liver	pale, diffuse, severe
		Spleen	decreased size, diffuse, moderate

Death code : M2 (Natural death)

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REDACTED AS TO TRADE NAMES

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 4)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#		TISSUE	Gross observations
5M	7	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
			Spleen	decreased size, diffuse, moderate
6F	15	M2	Liver	pale, diffuse, moderate
			Spleen	decreased size, diffuse, severe
7F	8	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
8F	10	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Skin treatment area	edema, diffuse, moderate
			Spleen	decreased size, diffuse, moderate

Death code : M2 (Natural death)

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 5)
(individual)

Dead or agonal sacrificed an.

Dose (mg/kg) 2000

An#	Death day/code#		TISSUE	Gross observations
8F	10	M2	Stomach	congestion, diffuse, moderate
9F	13	M2	Liver	pale, diffuse, moderate
10F	15	M2	Kidneys	medulla, congestion, diffuse, moderate
			Liver	pale, diffuse, severe
			Spleen	decreased size, diffuse, severe

Death code : M2 (Natural death)

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Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 6)
(individual)

Final killing

Dose (mg/kg) 200

An#	Death day	T I S S U E	Gross observations
31M	16	General observation	no macroscopically appreciable lesions
32M	16	General observation	no macroscopically appreciable lesions
33M	16	General observation	no macroscopically appreciable lesions
34M	16	General observation	no macroscopically appreciable lesions
35M	16	General observation	no macroscopically appreciable lesions

Test article: [redacted]
Title : Acute dermal toxicity study in rats
RBM exp. : 970593

APPENDIX 3. - Gross pathology examination (p. 7)
(individual)

Final killing

Dose (mg/kg) 500

An#	Death day	T I S S U E	Gross observations
22M	16	Liver	increased size, diffuse, moderate
23M	16	Liver	increased size, diffuse, moderate
24M	16	Liver	increased size, diffuse, moderate